

# SEQUENCE LISTING

<110> . INSERTM

5. <120> Novel membrane-bound NEP II metalloprotease and the use thereof for screening inhibitors useful in therapy

<130> BET 99/0150

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<140>-

<141>

<150> FR/9804389

15 <151> 1998-04-08

<160> 29

<170> PatentIn Ver.. 2.1

20

 $\langle 210 \rangle$  1

<211> 2765

<212> DNA

<213> Rattus rattus

25 . <220>

<221> CDS

$$\langle 222 \rangle \quad (107) \quad \dots \quad (2428)$$

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Met Gly Lys

TCG GAG AGC TCA GTG GGG ATG ATG GAG AGA GCG GAC AAC TGT GGG AGG 163  
Ser Glu Ser Ser Val Gly Met Met Glu Arg Ala Asp Asn Cys Gly Arg  
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AGG CGC CTA GGC TTC GTG GAG TGT GGG CTG CTG GTA CTG CTG ACA CTG 211  
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20 25 30 35

|     |     |         |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| CTG | TTG | ATG     | GGG | GCC | ATA | GTG | ACT | CTG | GGT | GTC | TTC | TAC | AGC | ATG | GGG | 259 |
| Leu | Leu | Met-Gly | Ala | Ile | Val | Thr | Leu | Gly | Val | Phe | Tyr | Ser | Ile | Gly |     |     |
|     |     |         | 40  |     |     |     |     |     | 45  |     |     |     |     | 50  |     |     |
| AAG | CAG | GTG     | CCC | CTC | TTA | AAT | AGC | CTG | CTG | CAC | GTC | TCC | CGG | CAT | GAG | 307 |
| Lys | Gln | Leu     | Pro | Leu | Leu | Asn | Ser | Leu | Leu | His | Val | Ser | Arg | His | Glu |     |
|     |     |         | 55  |     |     |     |     | 60  |     |     |     |     | 65  |     |     |     |
| AGG | ACG | GTT     | GTA | AAA | CGA | GTC | CTC | AGA | GAT | TCA | TCG | CAG | AAG | AGT | GAC | 355 |
| Arg | Thr | Val     | Val | Lys | Arg | Val | Leu | Arg | Asp | Ser | Ser | Gln | Lys | Ser | Asp |     |
|     |     |         | 70  |     |     |     | 75  |     |     |     |     | 80  |     |     |     |     |
| ATC | TGT | ACT     | ACC | CCA | AGC | TGC | GTG | ATA | GCA | GCT | GCC | AGA | ATC | CTC | CAG | 403 |
| Ile | Cys | Thr     | Thr | Pro | Ser | Cys | Val | Ile | Ala | Ala | Ala | Arg | Ile | Leu | Gln |     |
|     | 85  |         |     |     |     | 90  |     |     |     |     | 95  |     |     |     |     |     |
| AAC | ATG | GAC     | CAG | TCA | AAG | AAA | CCC | TGC | GAC | AAC | TTC | TAT | CAG | TAT | GCT | 451 |
| Asn | Met | Asp     | Gln | Ser | Lys | Lys | Pro | Cys | Asp | Asn | Phe | Tyr | Gln | Tyr | Ala |     |
| 100 |     |         |     |     | 105 |     |     |     |     | 110 |     |     |     |     | 115 |     |
| TGC | GGG | GGC     | TGG | CTA | CGG | CAC | CAT | GTG | ATC | CCC | GAG | ACC | AAC | TCC | AGA | 499 |
| Cys | Gly | Gly     | Trp | Leu | Arg | His | His | Val | Ile | Pro | Glu | Thr | Asn | Ser | Arg |     |
|     |     |         |     | 120 |     |     |     |     | 125 |     |     |     |     | 130 |     |     |
| TAC | AGC | GTC     | TTT | GAC | ATC | CTT | CGG | GAT | GAG | CTG | GAG | GTC | ATC | CTC | AAA | 547 |
| Tyr | Ser | Val     | Phe | Asp | Ile | Leu | Arg | Asp | Glu | Leu | Glu | Val | Ile | Leu | Lys |     |
|     |     |         | 135 |     |     |     |     | 140 |     |     |     |     | 145 |     |     |     |
| GGG | GTG | CTG     | GAG | GAT | TCC | TCT | GTC | CAG | CAC | CGC | CCA | GCT | GTG | GAG | AAG | 595 |
| Gly | Val | Leu     | Glu | Asp | Ser | Ser | Val | Gln | His | Arg | Pro | Ala | Val | Glu | Lys |     |
|     |     | 150     |     |     |     |     | 155 |     |     |     |     | 160 |     |     |     |     |
| GCC | AAG | ACA     | CTG | TAC | CGC | TCC | TGC | ATG | AAC | CAG | AGT | GTG | ATA | GAG | AAG | 643 |
| Ala | Lys | Thr     | Leu | Tyr | Arg | Ser | Cys | Met | Asn | Gln | Ser | Val | Ile | Glu | Lys |     |
|     | 165 |         |     |     |     | 170 |     |     |     |     | 175 |     |     |     |     |     |
| AGA | GAC | TCT     | GAG | CCC | CTG | CTG | AAC | GTC | TTA | GAT | ATG | ATA | GGA | GGT | TGG | 691 |
| Arg | Asp | Ser     | Glu | Pro | Leu | Leu | Asn | Val | Leu | Asp | Met | Ile | Gly | Gly | Trp |     |
| 180 |     |         |     |     | 185 |     |     |     |     | 190 |     |     |     |     | 195 |     |
| CCT | GTA | GCC     | ATG | GAC | AAG | TGG | AAT | GAG | ACC | ATG | GGC | CCC | AAG | TGG | GAA | 733 |

| Pro | Val | Ala | Met | Asp | Lys | Trp | Asn | Glu | Thr | Met | Gly | Pro | Lys | Trp | Glu |      |
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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 200 |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 205 |      |
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| CTG | GAG | CGG | CAG | TTG | GCT | GTG | TTG | AAC | TCG | CAG | TTC | AAC | AGG | CGC | GTC | 787  |
| Leu | Glu | Arg | Gln | Leu | Ala | Val | Leu | Asn | Ser | Gln | Phe | Asn | Arg | Arg | Val |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 215 |      |
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| CTC | ATC | GAC | CTC | TTT | ATC | TGG | AAT | GAT | GAC | CAG | AAC | TCC | AGC | CGG | CAC | 835  |
| Leu | Ile | Asp | Leu | Phe | Ile | Trp | Asn | Asp | Asp | Gln | Asn | Ser | Ser | Arg | His |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 230 |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 235 |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 240 |      |
| GTC | ATC | TAC | ATA | GAC | CAG | CCC | ACC | TTG | GGC | ATG | CCC | TCC | CGG | GAG | TAC | 883  |
| Val | Ile | Tyr | Ile | Asp | Gln | Pro | Thr | Leu | Gly | Met | Pro | Ser | Arg | Glu | Tyr |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 245 |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 250 |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 255 |      |
| TAT | TTT | AAG | GAA | GAC | AGC | CAC | CGG | GTA | CGG | GAA | GCC | TAC | CTG | CAG | TTT | 931  |
| Tyr | Phe | Lys | Glu | Asp | Ser | His | Arg | Val | Arg | Glu | Ala | Tyr | Leu | Gln | Phe |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 260 |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 265 |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 270 |      |
| ATG | ACA | TCA | GTG | GCC | ACT | ATG | CTG | AGG | AGA | GAC | CTG | AAC | CTG | CCC | GGG | 979  |
| Met | Thr | Ser | Val | Ala | Thr | Met | Leu | Arg | Arg | Asp | Leu | Asn | Leu | Pro | Gly |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 280 |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 285 |      |
| GAG | ACC | GAT | TTG | GTG | CAG | GAG | GAA | ATG | GCA | CAG | GTG | CTG | CAT | CTG | GAG | 1027 |
| Glu | Thr | Asp | Leu | Val | Gln | Glu | Glu | Met | Ala | Gln | Val | Leu | His | Leu | Glu |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 295 |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 300 |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 305 |      |
| ACA | CAT | CTG | GCC | AAC | GCC | ACG | GTC | CCC | CAG | GAG | AAA | AGG | CAT | GAT | GTC | 1075 |
| Thr | His | Leu | Ala | Asn | Ala | Thr | Val | Pro | Gln | Glu | Lys | Arg | His | Asp | Val |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 310 |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 315 |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 320 |      |
| ACC | GCC | CTG | TAT | CAC | CGA | ATG | GGC | CTG | GAG | GAG | CTG | CAG | GAA | AGG | TTT | 1123 |
| Thr | Ala | Leu | Tyr | His | Arg | Met | Gly | Leu | Glu | Glu | Leu | Gln | Glu | Arg | Phe |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 325 |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 330 |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 335 |      |
| GGT | CTG | AAG | GGG | TTT | AAC | TGG | ACT | CTC | TTC | ATA | CAA | AAC | GTG | CTG | TCT | 1171 |
| Gly | Leu | Lys | Gly | Phe | Asn | Trp | Thr | Leu | Phe | Ile | Gln | Asn | Val | Leu | Ser |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 340 |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 345 |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 350 |      |
| TCT | GTG | CAA | GTT | GAG | CTG | CTC | CCG | AAT | GAG | GAG | GTG | GTG | GTC | TAT | GGC | 1219 |
| Ser | Val | Gln | Val | Glu | Leu | Leu | Pro | Asn | Glu | Glu | Val | Val | Val | Tyr | Gly |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 360 |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 365 |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 370 |      |

|   |      |
|---|------|
| ATC CCC TAC CTG GAG AAT CTT GAG GAG ATC ATT GAC GTC TTC CCA GCA | 1267 |
| Ile Pro Tyr Leu Glu Asn Leu Glu Glu Ile Ile Asp Val Phe Pro Ala |      |
| 375 380 385   |      |
| CAG ACC TTG CAA AAC TAC CTG GTG TGG CGC CTG GTG CTA GAT CGC ATC | 1315 |
| Gln Thr Leu Gln Asn Tyr Leu Val Trp Arg Leu Val Leu Asp Arg Ile |      |
| 390 395 400   |      |
| GGC AGC CTG AGC CAG AGA TTC AAA GAA GCG CGT GTG GAC TAC CGC AAG | 1363 |
| Gly Ser Leu Ser Gln Arg Phe Lys Glu Ala Arg Val Asp Tyr Arg Lys |      |
| 405 410 415   |      |
| GCG CTG TAC GGT ACA ACC ATG GAG GAA GTA CGC TGG CGG GAG TGT GTC | 1411 |
| Ala Leu Tyr Gly Thr Thr Met Glu Glu Val Arg Trp Arg Glu Cys Val |      |
| 420 425 430 435   |      |
| AGC TAT GTC AAC AGC AAC ATG GAG AGT GCC GTG GGC TCC CTC TAC ATC | 1459 |
| Ser Tyr Val Asn Ser Asn Met Glu Ser Ala Val Gly Ser Leu Tyr Ile |      |
| 440 445 450   |      |
| AAG CGG GCC TTC TCC AAG GAC AGC AAG AGC ATA GTC AGT GAG CTT ATC | 1507 |
| Lys Arg Ala Phe Ser Lys Asp Ser Lys Ser Ile Val Ser Glu Leu Ile |      |
| 455 460 465   |      |
| GAG AAG ATA CGG TCC GTG TTT GTG GAT AAC CTG GAC GAG TTG AAC TGG | 1555 |
| Glu Lys Ile Arg Ser Val Phe Val Asp Asn Leu Asp Glu Leu Asn Trp |      |
| 470 475 480   |      |
| ATG GAT GAG GAA TCC AAG AAA AAG GCC CAG GAA AAG GCC TTG AAT ATC | 1603 |
| Met Asp Glu Glu Ser Lys Lys Lys Ala Gln Glu Lys Ala Leu Asn Ile |      |
| 485 490 495   |      |
| CGG GAA CAG ATC GGC TAC CCT GAC TAC ATT TTG GAA GAC AAT AAC AGA | 1651 |
| Arg Glu Gln Ile Gly Tyr Pro Asp Tyr Ile Leu Glu Asp Asn Asn Arg |      |
| 500 505 510 515   |      |
| CAC CTG GAT GAG GAA TAC TCC AGT CTG ACT TTC TCA GAG GAC CTG TAT | 1699 |
| His Leu Asp Glu Glu Tyr Ser Ser Leu Thr Phe Ser Glu Asp Leu Tyr |      |
| 520 525 530   |      |
| TTT GAG AAC GGG CTT CAG AAC CTC AAG AAC AAT GCC CAA AGG AGC CTC | 1747 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|--|
| Phe | Glu | Asn | Gly | Leu | Gln | Asn | Leu | Lys | Asn | Asn | Ala | Gln | Arg | Ser | Leu |      |  |
|     |     | -   | 535 |     |     |     |     | 540 |     |     |     |     | 545 |     |     |      |  |
| AAG | AAA | CTT | CGG | GAA | AAG | GTG | GAC | CAG | AAT | CTC | TGG | ATC | ATT | GGG | GCT | 1795 |  |
| Lys | Lys | Leu | Arg | Glu | Lys | Val | Asp | Gln | Asn | Leu | Trp | Ile | Ile | Gly | Ala |      |  |
|     |     | 550 |     |     |     |     | 555 |     |     |     |     | 560 |     |     |     |      |  |
| GCA | GTG | GTC | AAT | GCA | TTC | TAC | TCC | CCA | AAC | AGA | AAC | CTG | ATC | GTC | TTT | 1843 |  |
| Ala | Val | Val | Asn | Ala | Phe | Tyr | Ser | Pro | Asn | Arg | Asn | Leu | Ile | Val | Phe |      |  |
|     | 565 |     |     |     |     | 570 |     |     |     |     | 575 |     |     |     |     |      |  |
| CCA | GCG | GGG | ATC | CTC | CAG | CCA | CCC | TTC | TTC | AGC | AAG | GAC | CAA | CCA | CAG | 1891 |  |
| Pro | Ala | Gly | Ile | Leu | Gln | Pro | Pro | Phe | Phe | Ser | Lys | Asp | Gln | Pro | Gln |      |  |
| 580 |     |     |     |     | 585 |     |     |     |     | 590 |     |     |     |     | 595 |      |  |
| GCC | TTG | AAT | TTC | GGG | GGC | ATC | GGG | ATG | GTG | ATT | GGA | CAC | GAG | ATC | ACA | 1939 |  |
| Ala | Leu | Asn | Phe | Gly | Gly | Ile | Gly | Met | Val | Ile | Gly | His | Glu | Ile | Thr |      |  |
|     |     |     | 600 |     |     |     |     |     | 605 |     |     |     |     | 610 |     |      |  |
| CAC | GGC | TTT | GAT | GAT | AAC | GGT | CGG | AAC | TTT | GAC | AAG | AAT | GGC | AAC | ATG | 1987 |  |
| His | Gly | Phe | Asp | Asp | Asn | Gly | Arg | Asn | Phe | Asp | Lys | Asn | Gly | Asn | Met |      |  |
|     |     | 615 |     |     |     |     | 620 |     |     |     |     |     | 625 |     |     |      |  |
| CTG | GAC | TGG | TGG | AGC | AAC | TTC | TCG | GCC | CGG | CAC | TTC | CGA | CAG | CAG | TCA | 2035 |  |
| Leu | Asp | Trp | Trp | Ser | Asn | Phe | Ser | Ala | Arg | His | Phe | Arg | Gln | Gln | Ser |      |  |
|     |     | 630 |     |     |     |     | 635 |     |     |     |     | 640 |     |     |     |      |  |
| CAG | TGT | ATG | ATT | TAT | CAG | TAC | AGC | AAC | TTC | TCT | TGG | GAA | CTA | GCA | GAC | 2083 |  |
| Gln | Cys | Met | Ile | Tyr | Gln | Tyr | Ser | Asn | Phe | Ser | Trp | Glu | Leu | Ala | Asp |      |  |
|     | 645 |     |     |     |     | 650 |     |     |     |     | 655 |     |     |     |     |      |  |
| AAC | CAG | AAT | GTG | AAC | GGA | TTC | AGC | ACC | CTC | GGG | GAG | AAC | ATC | GCC | GAC | 2131 |  |
| Asn | Gln | Asn | Val | Asn | Gly | Phe | Ser | Thr | Leu | Gly | Glu | Asn | Ile | Ala | Asp |      |  |
| 660 |     |     |     |     | 665 |     |     |     |     | 670 |     |     |     |     | 675 |      |  |
| AAC | GSC | GGT | GTG | CGG | CAG | GCA | TAC | AAG | GCT | TAC | CTA | CAG | TGG | CTA | GCT | 2179 |  |
| Asn | Gly | Gly | Val | Arg | Gln | Ala | Tyr | Lys | Ala | Tyr | Leu | Gln | Trp | Leu | Ala |      |  |
|     |     |     | 680 |     |     |     |     | 685 |     |     |     |     |     | 690 |     |      |  |
| GAA | GSC | GGC | AGA | GAC | CAG | AGA | CTG | CCG | GGA | CTG | AAC | CTG | ACC | TAT | GCT | 2227 |  |
| Glu | Gly | Gly | Arg | Asp | Gln | Arg | Leu | Pro | Gly | Leu | Asn | Leu | Thr | Tyr | Ala |      |  |
|     |     | 695 |     |     |     |     |     | 700 |     |     |     |     | 705 |     |     |      |  |

Met Gly Lys Ser Glu Ser Ser Val Gly Met Met Glu Arg Ala Asp Asn.  
1 5 10 15

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Cys | Gly | Arg | Arg | Arg | Leu | Gly | Phe | Val | Glu | Cys | Gly | Leu | Leu | Val | Leu |  |
|     |     |     |     | 20  |     |     |     | 25  |     |     |     |     |     | 30  |     |  |
| Leu | Thr | Leu | Leu | Leu | Met | Gly | Ala | Ile | Val | Thr | Leu | Gly | Val | Phe | Tyr |  |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |  |
| Ser | Ile | Gly | Lys | Gln | Leu | Pro | Leu | Leu | Asn | Ser | Leu | Leu | His | Val | Ser |  |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |  |
| Arg | His | Glu | Arg | Thr | Val | Val | Lys | Arg | Val | Leu | Arg | Asp | Ser | Ser | Gln |  |
|     | 65  |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |  |
| Lys | Ser | Asp | Ile | Cys | Thr | Thr | Pro | Ser | Cys | Val | Ile | Ala | Ala | Ala | Arg |  |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     |     | 95  |  |
| Ile | Leu | Gln | Asn | Met | Asp | Gln | Ser | Lys | Lys | Pro | Cys | Asp | Asn | Phe | Tyr |  |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |  |
| Gln | Tyr | Ala | Cys | Gly | Gly | Trp | Leu | Arg | His | His | Val | Ile | Pro | Glu | Thr |  |
|     | 115 |     |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |  |
| Asn | Ser | Arg | Tyr | Ser | Val | Phe | Asp | Ile | Leu | Arg | Asp | Glu | Leu | Glu | Val |  |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |  |
| Ile | Leu | Lys | Gly | Val | Leu | Glu | Asp | Ser | Ser | Val | Gln | His | Arg | Pro | Ala |  |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |  |
| Val | Glu | Lys | Ala | Lys | Thr | Leu | Tyr | Arg | Ser | Cys | Met | Asn | Gln | Ser | Val |  |
|     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |  |
| Ile | Glu | Lys | Arg | Asp | Ser | Glu | Pro | Leu | Leu | Asn | Val | Leu | Asp | Met | Ile |  |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |  |
| Gly | Gly | Trp | Pro | Val | Ala | Met | Asp | Lys | Trp | Asn | Glu | Thr | Met | Gly | Pro |  |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |  |
| Lys | Trp | Glu | Leu | Glu | Arg | Gln | Leu | Ala | Val | Leu | Asn | Ser | Gln | Phe | Asn |  |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |  |
| Arg | Arg | Val | Leu | Ile | Asp | Leu | Phe | Ile | Trp | Asn | Asp | Asp | Gln | Asn | Ser |  |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |  |

[illegible]



Asn Arg Cys Arg Ile Trp  
770

10

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15

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| $\langle 212 \rangle$ | DNA |

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